Grain dormancy and light quality effects on germination in the model grass *Brachypodium distachyon*

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Summary

- Lack of grain dormancy in cereal crops such as barley and wheat is a common problem affecting farming areas around the world, causing losses in yield and quality because of preharvest sprouting. Control of seed or grain dormancy has been investigated extensively using various approaches in different species, including Arabidopsis and cereals. However, the use of a monocot model plant such as *Brachypodium distachyon* presents opportunities for the discovery of new genes related to grain dormancy that are not present in modern commercial crops.
- In this work we present an anatomical description of the *Brachypodium* caryopsis, and we describe the dormancy behaviour of six common diploid *Brachypodium* inbred genotypes. We also study the effect of light quality (blue, red and far-red) on germination, and analyse changes in abscisic acid levels and gene expression between a dormant and a non-dormant *Brachypodium* genotype.
- Our results indicate that different genotypes display high natural variability in grain dormancy and that the characteristics of dormancy and germination are similar to those found in other cereals.
- We propose that *Brachypodium* is an ideal model for studies of grain dormancy in grasses and can be used to identify new strategies for increasing grain dormancy in crop species.

Introduction

Crop plants have been modified during their domestication and subsequent breeding in order to improve their agronomic characteristics, and this selective pressure has generated a new set of traits displayed by crop plants known as the Domestication Syndrome (Harlan, 1975). In cereal crops the most important changes compared with wild-type ancestors are the suppression of spikelet shattering, increased grain size, a reduction in the number of tillers and the loss of grain dormancy. In fact, the selection for grains that display a high germination rate and that show a predictable and synchronous germination is a major feature of the Domestication Syndrome in cereals. This has generated many modern cereal cultivars that have low grain dormancy thereby rendering them susceptible to preharvest sprouting. Preharvest sprouting is a recurrent problem common in many cereal cropping areas in the world, especially those affected by moist weather before harvest (Gubler *et al.*, 2005).

Many different approaches including mutagenesis, proteomics, metabolomics or quantitative trait loci (QTL) and gene expression analysis have been used to study dormancy in model plants such as Arabidopsis (reviewed in Barrero *et al.*, 2010). This has led to good progress in our understanding of molecular and hormonal mechanisms that regulate dormancy in Arabidopsis seeds. While it is recognized that model plants such as Arabidopsis share some dormancy pathway components (e.g. ABI3 and VP1) with cereals, it has become apparent that there are also major differences in terms of responses to environmental cues such as light (Simpson, 1990; Goggin *et al.*, 2008; Gubler *et al.*, 2008) as well as contributions of different seed tissues to dormancy. The use of a cereal model plant such as rice (*Oryza sativa*) with a sequenced genome could provide a suitable system for studying dormancy in cereals and there has been recent progress toward identifying dormancy genes in high dormancy rice species such as weedy rice (Gu *et al.*, 2010; Ye *et al.*, 2010). However, the use of rice has been hampered by the overall low dormancy present in most rice lines. A few attempts using wild ancestors of wheat (*Triticum aestivum*) in dormancy studies have been made (Imtiaz *et al.*, 2008), but these analyses have proved to be a challenge because of the large genome sizes and the lack of molecular tools.

The wild grass, *Brachypodium distachyon*, has emerged as an attractive diploid model for temperate cereals such as wheat and barley (*Hordeum vulgare*) (Draper *et al.*, 2001; Garvin *et al.*, 2008). Its small genome size and short life-cycle make it ideal for gene discovery approaches in an undomesticated member of the Pooideae, and presents opportunities for the discovery of new genes or alleles with a role in grain dormancy that are not present in modern commercial crops. In this paper we present a detailed anatomical description of the *Brachypodium* caryopsis and describe the dormancy characteristics of this species. Using six phylogenetically diverse genotypes, we have described their germination, after-ripening requirements, husk effects, and the effect of light quality in promoting dormancy or germination. We have also analysed the ABA levels and gene expression changes comparing a dormant with a non-dormant *Brachypodium* genotype. Our results indicate that *Brachypodium* represents an excellent model for studies on grain dormancy in temperate cereals.

Materials and Methods

Plant lines and growth conditions

The genotypes used in this work were kindly donated by Dr Iain Wilson, who obtained them from Drs David Garvin and John Vogel. Plants were grown in 11 cm diameter pots with soil containing seed raising mix (Debco, Melbourne, VIC, Australia) supplemented with 2 g l^{-1} Osmocote, at a density of *c.* 15 plants per pot. Controlled environment rooms were used with a 20-h photoperiod for accelerating flowering, a light intensity of 180 μmol m^{-2} s^{-1} and a temperature of 20°C. Grains from each genotype were harvested in bulk when the spikes were dry, threshed by hand to prevent damage to the husk and embryo and dried for 3 d in the laboratory. Subsequently, half of the grains were stored at −20°C in order to retain dormancy and the rest were after-ripened at 37°C (or at 20°C for results in Fig. 6a). Genotypes Bd18-1 and Bd1-1 were grown for 7 d and then vernalized for 16 wk at 4°C to promote flowering.

Embryo anatomy

Dry grains were dissected either dry or after hydration for 36 h on moist filter paper in the dark in sealed Petri dishes. For light microscopy, dry and hydrated grains were fixed in 2% formaldehyde, 0.1% glutaraldehyde with 0.01% Tween-20 in 50 mM 25 mM piperazine-*N*, *N*′-bis (2-ethanesulfonic acid) (PIPES) buffer, pH 6.8 for 2 h at room temperature with vacuum infiltration, and then left in fixative overnight at 4°C. After several buffer rinses, grains were dehydrated in an ethanol series over 1 wk, and then infiltrated with LR White resin (medium grade) (London Resin Company Ltd, London, UK) over 3 wk and stored in 100% resin for a further 2–4 wk before embedding. Sections 1–2 μm thick were stained with 0.1% aqueous toluidine blue. For scanning electron microscopy, grains (either whole or dissected to reveal details of dry or imbibed embryos) were fixed in 70% ethanol, dehydrated and critical-point dried, then observed with a Zeiss EVO LS15 SEM (Carl Zeiss Australasia, Sydney, NSW, Australia). Images were processed (sharpened, brightness and contrast adjusted) and assembled using a combination of Automontage Essentials (Syncroscopy, Frederick, MD, USA) and Photoshop CS5 (Adobe, San Jose, CA, USA).

Germination assays

For imbibition experiments, replicate sets of 20 dormant or after-ripened grains were placed on 9-cm plastic Petri dishes containing two 9-cm Whatman number 1 filter papers and 4 ml of distilled water. The plates were sealed with Parafilm and incubated at 20°C in continuous white fluorescent light with an intensity of 150 μmol m^{-2} s^{-1} over the waveband 400–700 nm, using TLD 36W/865 tubes (Phillips, North Ryde, NSW, Australia), or wrapped in two layers of aluminium foil for darkness, for 10 d. Germination was scored every 24 h, and grains were scored as germinated when the coleorhiza had emerged beyond the husk (Barrero *et al.*, 2009). Experiments were performed in triplicate for each genotype examined. The grain used in each experiment was harvested from the same batch of plants, which were grown at the same time and in the same environment. Nongerminated grains were stratified for 1 wk at 4°C in order to break the dormancy and then returned to the germination chamber in darkness to check their viability.

Imbibitions under different light quality regimes were performed using monochromatic LEDs in a light-tight box with temperature maintained at 20°C, as described previously for barley (Gubler *et al.*, 2008). Intensities of blue, red, and far-red light were 38, 11 and 63 μmol m^{-2} s^{-1}, respectively, measured with a Licor LI-1800 spectroradiometer (Licor, Lincoln, Nebraska, USA). For daily counting of germination a safe green-light LED torch (1.7 μmol m^{-2} s^{-1}) was used.

Quantification of ABA

The ABA content of dissected dry or imbibed embryos was measured using a Phytodetek Competitive enzyme-linked immunosorbent assay (ELISA) kit (Agdia, Elkhart, IN, USA). Samples of 25 embryos were dissected using a microscope and frozen on dry ice. The ABA extractions were performed in 80% methanol as described previously (Gubler *et al.*, 2008). Three biological replicates were assayed.

RNA extraction and quantitative real-time PCR

RNA was prepared from embryos isolated from dry and imbibed grains using a method adapted from the hexadecyltrimethylammonium procedure described by Chang *et al.* (1993). Twenty-five embryos were frozen in liquid nitrogen and then homogenized using one stainless steel ball bearing and a tissue lyser (Qiagen) at 30 cycles s^{-1}. One millilitre of 65°C RNA extraction buffer containing 2% hexadecyltrimethylammonium (w : v) was added to the ground powder. The RNA was treated with DNase on mini RNeasy columns (Qiagen), and its quality was assessed on a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). A total of 2 μg of total RNA was then used to synthesize cDNA using SuperScript III (Invitrogen) following the supplier's recommendations in 20 μl reactions.

Barley sequences for *HvNCED1*, *HvNCED2*, *HvABA8′OH-1* and *HvABA8′OH-2* were used for searching the *Brachypodium* genome using the BLAST tool available at http://www.Brachypodium.org.

The genes for Bradi4g01440 (*BdNCED1*), Bradi1g13760 (*BdNCED2*), Bradi3g52660 (*BdABA8′OH-1*) and Bradi3g38150 9 (*BdABA8′OH-2*) were identified by sequence homology, showing 77%, 89%, 95% and 72% identity, respectively, to their barley homologues. Specific primers designed were: *BdNCED1*, 5′-GATTTCGACGGCCAGCTCTC-3′, 5′-AGCTCGATTTCCA CGTCGTTC-3′; *BdNCED2*, 5′-TTTGACGGGCAGCTCGA GTG-3′, 5′-TCTCGACGTCCGCCGACTTG-3′; *BdABA8′OH-1*, 5′-TCCATCCTCTCCTTCACCTTC-3′, 5′-TCGAAT CGGGAAGGATCAAACT-3′; and *BdABA8′OH-2*, 5′-ACCAG ATCGCCGACAACGTC-3′, 5′- CTCGTACGTCGCCATTT GCTC-3′. cDNA was diluted 100-fold, and 10 μl was used in 20 μl PCR samples with Platinum *Taq* (Invitrogen) and SYBR Green (Invitrogen). Reactions were run on a Rotor-gene 3000A real-time PCR machine (Corbett, Doncaster, VIC, Australia), and data were analysed with ROTOR-GENE software using the comparative quantification tool. Following Hong *et al.* (2008) three genes, *UBQ18* (Bradi4g00660), *ACT7* (Bradi1g02580) and *GAPDH* (Bradi3g14120), were tested as internal controls and finally only *GAPDH* (primers 5′-CTGCACCACTAACTGTCTTGC-3′ and 5′-GGAATGATGTTAAAGCTTGCAGC-3′) was used to normalize gene expression in our experiments as it was the most stable during grain germination (data not shown). Three biological replicates were performed for each experiment.

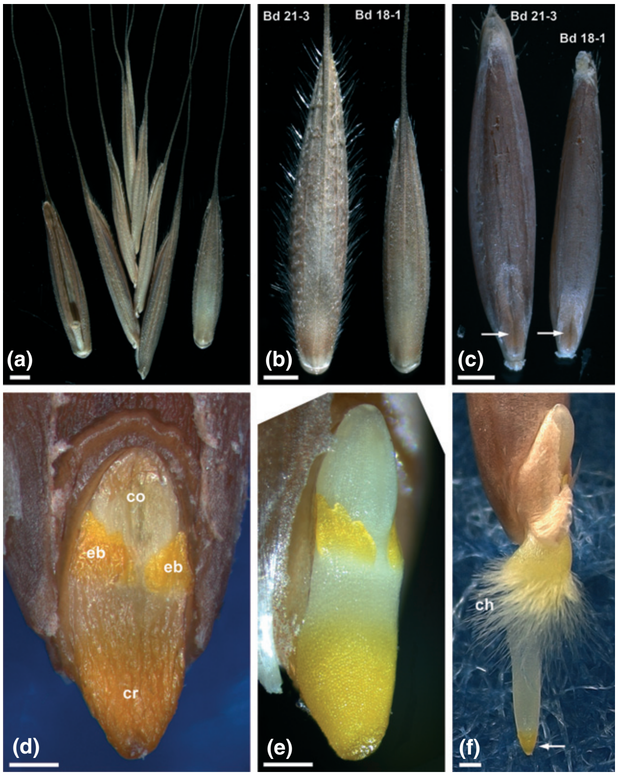

Fig. 1 *Brachypodium* grain and embryo morphology. (a) Spike from genotype Bd18-1 showing spikelet arrangement and ventral (left) and dorsal (right) surfaces of the grains. (b) Dorsal surface of intact grains showing smaller size of grain from genotype Bd18-1, and abundant long hairs on grain from genotype Bd21-3. (c) After removal of the husk, the embryos are visible. Arrows indicate the position of the embryos. (d) Dry embryo from genotype Bd21-3 showing corrugated surfaces of the white coleoptile (co), yellow epiblasts (eb) and coleorhiza (cr). (e) After 24 h imbibition, the surfaces of embryo tissues are smooth, and the coleorhiza and coleoptile have begun to elongate. (f) By 36–48 h, the coleorhiza is covered in dense hairs (ch) and the root emerges, with yellow root cap (arrow). Bars, (a–c) 1 mm, (d–f) 200 μm.

Results

Caryopsis anatomy

As previous work demonstrated the importance of the coleorhiza in regulation of dormancy and germination (Barrero *et al.*, 2009), we analysed *Brachypodium* embryo anatomy in detail, focusing on dry and imbibed grains from the most dormant genotype Bd18-1 and the least dormant genotype Bd21-3 (see later). As we previously detected no differences in anatomy between dormant and after-ripened barley grains (Barrero *et al.*, 2009), we used only after-ripened grains for the following analyses.

The grains of the two genotypes were similar in overall structure, with minor differences in size and shape and abundance of husk trichomes (Fig. 1a,b). Grains from Bd21-3 were, in general, larger than those from genotype Bd18-1, and had long hairs on the outer husk (lemma and palea). Embryos from Bd21-3 were also slightly larger than those from Bd18-1 (Figs 1c, 2a,b). Dry embryos from both genotypes had a distinct, yellow-pigmented coleorhiza and two yellow epiblasts, and had regular folds, especially in the coleorhiza (Fig. 1d). Upon imbibition, these folds disappeared (Figs 1e,f, 2a,b), and the yellow pigment in coleorhiza, epiblast (Fig. 1e,f) and emerging root cap (Fig. 1f) was prominent.

A further distinct feature of *Brachypodium* embryos was their single seminal root (Figs 1f, 2c,d and 3a), unlike barley which has one large central root with four or five less advanced seminal root primordia in the dry embryo (Barrero *et al.*, 2009). The coleorhiza formed a narrow tissue layer surrounding the single seminal root. Similar to barley embryos (Barrero *et al.*, 2009), longitudinal sections revealed a radial gradient in coleorhiza cell length from short epidermal cells to longer cells closest to the embryo root (Fig. 3b). However, these internal cells were tightly packed with small intercellular spaces, unlike in barley coleorhiza, and also tightly adhered to the enclosed root (Fig. 3a–c). Coleorhiza cells in the tip were small and isodiametric in shape, with some intercellular spaces (Fig. 3f); the epidermal cells were columnar in shape towards the tip (Fig. 3f). The epidermal cell walls appeared wrinkled in scanning electron microscopy (SEM) images of dry grains, and only slight gaps could be detected at cell junctions (Fig. 3f,h).

Upon imbibition, the coleorhiza and embryo root commenced rapid elongation, becoming highly vacuolated within 24–36 h. During this time, all cells of the coleorhiza also became somewhat separated (Fig. 3d,e), although the epidermal cells did not separate (Fig. 3g,i). No cell division was detected in coleorhiza cells. In contrast to barley, the cells of the coleorhiza tip elongated to three to four times their original length and expanded radially to protrude from the husk. At the outermost tip of the coleorhiza, a small protrusion of cells that could be seen in both dry and imbibed grain (Fig. 3a) linked it to the enclosing husk layer. The internal cells at the coleorhiza tip underwent mostly isodiametric

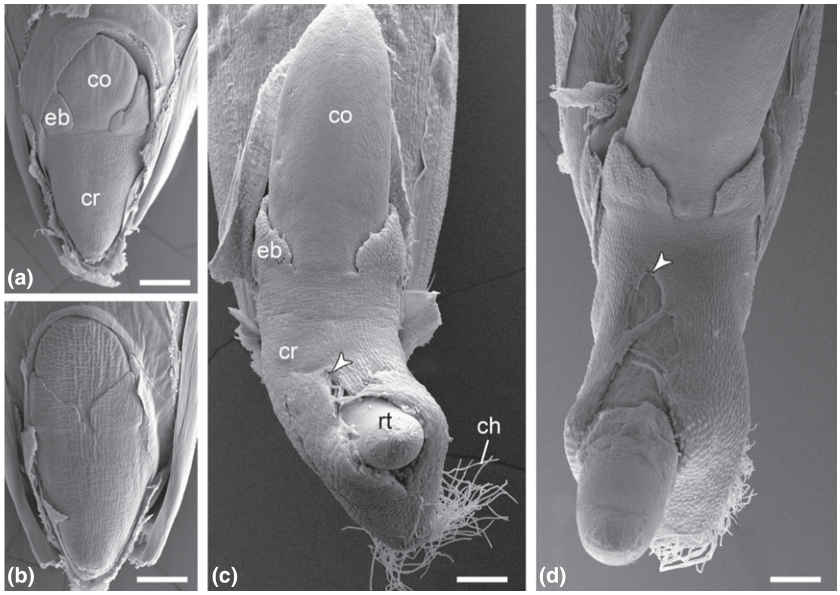

Fig. 2 Anatomy of *Brachypodium* embryos during germination. Scanning electron microscopy of dry (a,b) and 36 h-imbibed (c,d) Bd18-1 (a,c) and Bd21-3 (b,d) embryos. A single root emerges from a split in the side of the coleorhiza during germination (arrowheads, c and d). co, coleoptile; ch, coleorhiza hairs; cr, coleorhiza; eb, epiblast; rt, root. Bars, 200 μm.

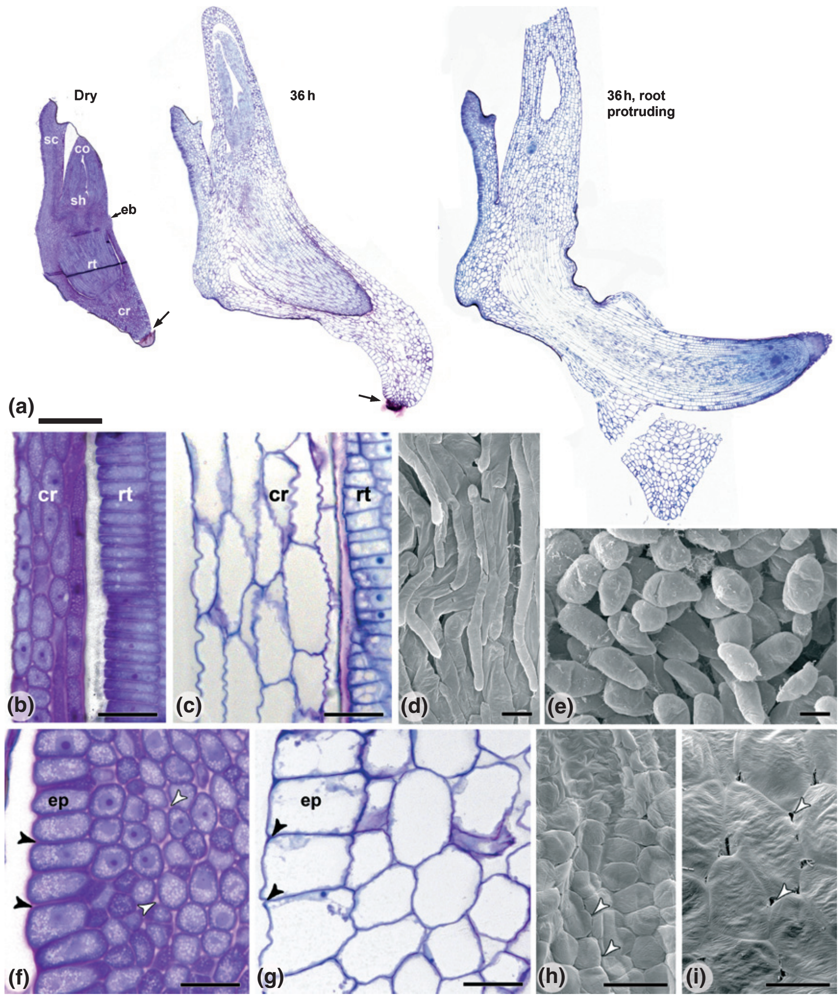

Fig. 3 Anatomy of *Brachypodium* embryos during germination in Bd18-1. Light microscope images of thin sections (a–c,f,g) and scanning electron microscopy (SEM) of coleorhiza cells adjacent to the root (d) and in the tip (e) of emerged coleorhiza, and on the outer coleorhiza surface of dry (h) and imbibed grain (i). (a) Sections of a dry embryo (left), 36-h imbibed embryo with elongated coleorhiza (centre) and imbibed embryo with emerging root (right); co, coleoptile; cr, coleorhiza; eb, epiblast; rt, root; sc, scutellum; sh, shoot. A small cluster of cells at the coleorhiza tip connects the coleorhiza to surrounding tissue (arrows). (b) In dry grain a thin layer of densely cytoplasmic coleorhiza cells (cr) surrounds the root (rt). (c) After 36 h imbibition these cells expand and become highly vacuolated. (d) SEM shows the highly elongated and separated coleorhiza cells adjacent to the root after imbibition (the root has been removed), and the separated cells in the tip of the coleorhiza (e). (f) In dry grain, cells towards the tip of the coleorhiza are isodiametric in shape and separated by small intercellular spaces (white arrowheads) while the epidermal cells are more columnar. (g) After 36 h of imbibition, coleorhiza tip cells expand isodiametrically and become highly vacuolated. The epidermal cells remain in close contact during germination, with minimal intercellular gaps found on the outer surface (black arrowheads in f and g). SEM shows these gaps between epidermal cells (in similar regions of the coleorhiza to those shown in f and g) in dry grain (arrowheads, h) and in germinated grain (arrowheads, i). Bars, (all images in a) 300 μm, (b–i) 20 μm.

expansion and became separated, with large airspaces (Fig. 3e,g). As in barley, the seminal root usually broke through longitudinal cracks in the coleorhiza rather than through the tip (Fig. 2c,d).

Germination, dormancy, and after-ripening behaviour

Grains from six different diploid *Brachypodium* inbred genotypes (Bd21-3, Bd2-3, Bd21, Bd3-1, Bd18-1 and Bd1-1; Table 1)

Table 1 Inbred *Brachypodium distachyon* genotypes used in this work

Inbred line	Origin	Vernalization	Dormancy
Bd1-1	Turkey	Yes	High
Bd2-3	Iraq	No	Low
Bd3-1	Iraq	No	Intermediate
Bd18-1	Turkey	Yes	High
Bd21	Iraq	No	Intermediate
Bd21-3	Iraq	No	Low

were harvested just after maturation (see the Materials and Methods section) and germination was scored without further after-ripening. Genotypes were chosen as they are phylogenetically diverse (Vogel *et al.*, 2009) and commonly used in research (Bd21 has been sequenced and Bd21-3 can be readily transformed; The International *Brachypodium* Initiative, 2010; Vogel & Hill, 2008). Grains from the six lines were imbibed in Petri dishes under continuous white light or in darkness, and germination was scored every day until day 10, after which very little new germination occurred. The germination time-courses of these grains were very different indicating high variability in grain dormancy between *Brachypodium* genotypes (Fig. 4). In the dark, genotypes Bd21-3 and Bd2-3 displayed the lowest level of dormancy, Bd18-1 and Bd1-1 displayed strong dormancy, and Bd21 and Bd3-1 displayed an intermediate dormancy. Germination rates under continuous light was lower than in darkness but Bd21-3 and Bd2-3 remained the least dormant and Bd18-1 and Bd1-1 the most dormant.

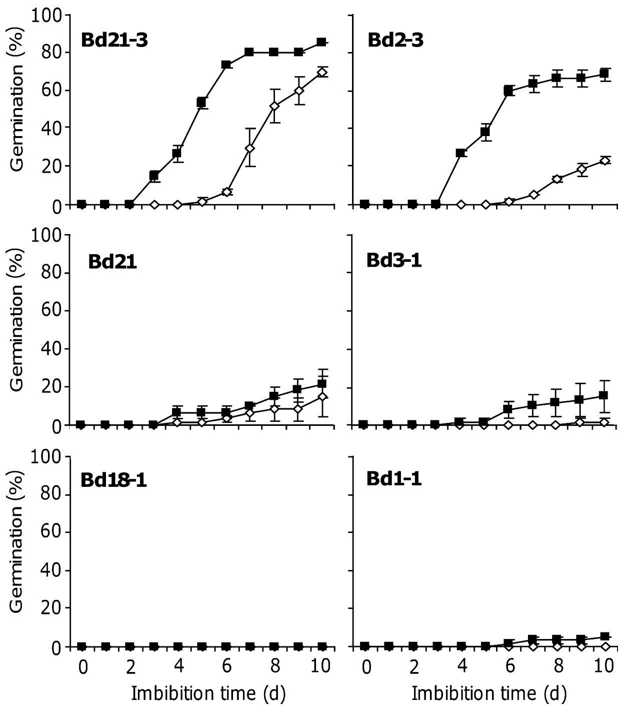

Fig. 4 Germination time courses of freshly-harvested grains, under continuous white light (open diamonds) or in darkness (closed squares). Grains from different *Brachypodium* genotypes were imbibed in water and germination was scored daily for 10 d. Measurements are averages of three replicates with error bars representing SE of the mean.

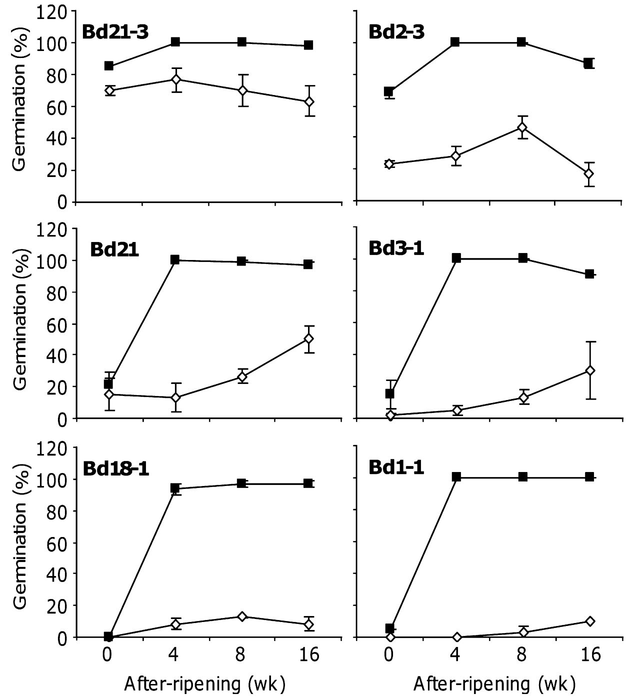

Fig. 5 Germination in white light (open diamonds) or darkness (closed squares) of grains after-ripened for different durations. After-ripened grain from different *Brachypodium* genotypes was imbibed in water and germination was scored at day 10. Measurements are averages of three replicates with error bars representing SE of the mean.

After this initial dormancy test, germination was scored again in grains from the same genotypes that were dry-stored for different durations, in order to determine the after-ripening time needed to break dormancy. Fig. 5 shows the germination % at day 10 of grains that were after-ripened for 0 (initial dormancy test), 4, 8 and 16 wk. In all cases the initial dark-dormancy disappeared very quickly, and after 4 wk of after-ripening the germination rates in all genotypes were close to 100%. By contrast, when dormancy was tested under continuous light the germination rate generally increased very slowly with after-ripening. In some cases the germination rates under continuous light reached a plateau, and dormancy was only broken after a long after-ripening period lasting between 6 and 12 months, depending on environmental temperature (data not shown). Dormancy was broken at a slower rate when after-ripened at 20°C compared with 37°C. For example, Bd18-1 reached a rate of *c.* 20% germination after 16 wk of after-ripening at 20°C, but when after-ripened at 37°C the germination rate increased to 80% (Fig. 6a). In both conditions a steady level of dormancy was reached and little change occurred between week 16 and week 20 (Fig. 6a), possibly owing to the acquisition of secondary dormancy.

Brachypodium produces grains that are covered by a husk (lemma and palea), as is the case for all wild grasses. In some species the presence of the husk has a major effect on grain dormancy, which disappears once the husk is removed (physical dormancy; Finch-Savage & Leubner-Metzger, 2006), indicating a very low level of embryo-based dormancy. To investigate the level of embryo-based dormancy in *Brachypodium*, the effect of husk removal was examined in the dormant genotype Bd18-1

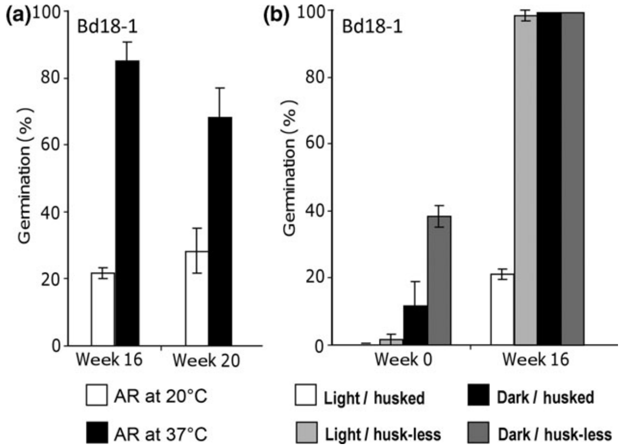

Fig. 6 Effects of temperature on after-ripening and of husk on dormancy in *Brachypodium*. (a) Bd18-1 grains were after-ripened for 16 wk or 20 wk at 20 or 37°C, and then imbibed in water. (b) Dormant and after-ripened Bd18-1 grains were imbibed in water undamaged or manually dehusked. Germination was scored at day 10. Measurements are averages of three replicates with error bars representing SE of the mean.

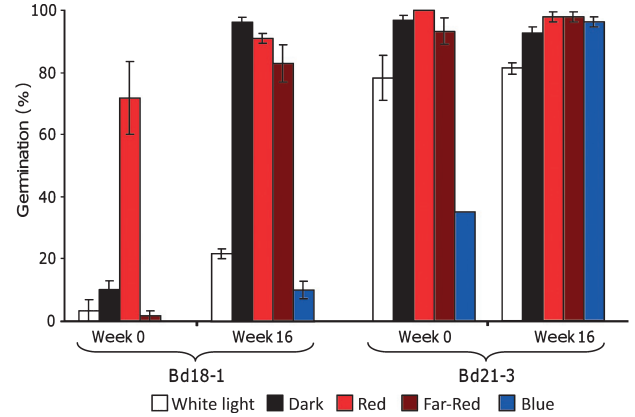

Fig. 7 Light quality effects on germination of dormant and after-ripened grains in *Brachypodium*. Dormant or after-ripened Bd18-1 and Bd21-3 grains were imbibed in water and then irradiated continuously with lights of different wavelengths. Germination was scored at day 10. Measurements are averages of three replicates with error bars representing SE of the mean.

using freshly-harvested grains and grains after-ripened for 16 wk. At week 0, both husked and huskless grains showed strong dormancy in the light. In darkness husked grains displayed *c.* 10% germination and huskless grains *c.* 40% (Fig. 6b). After 16 wk of after-ripening, both husked and huskless grains fully germinated in the dark (Fig. 6b). However, in the light, after-ripened husked grains displayed 20% germination while huskless grains germinated at almost 100%. These results suggest that the embryo contributes strongly to dormancy in *Brachypodium*.

Light quality effects on germination

Light is a very important environmental signal triggering germination or promoting dormancy, depending on the species. In Arabidopsis, white and red light promote germination, while in cereals such as barley and wheat, white and blue light promote dormancy (Gubler *et al.*, 2008). To allow comparison with domesticated grasses, we analysed the effect of light quality on dormancy and germination in *Brachypodium* using Bd18-1 (high dormancy) and Bd21-3 (low dormancy) (Fig. 7). In freshly-harvested (dormant) Bd18-1 grains, germination was very low under continuous white light, and was only slightly higher in darkness. Continuous red light strongly promoted germination (to 70%), while continuous far-red and blue strongly repressed germination below the level observed in darkness. In Bd18-1 grain after-ripened for 16 wk, germination rate was close to 100% in the dark, and under red or far-red. However, blue light still had a strong inhibitory effect.

Bd21-3 produces grains with weak dormancy at harvest and under white light germination was high (80%). Germination was also high in darkness, red, or far-red using both freshly-harvested and after-ripened grains (Fig. 7). However, in freshly-harvested grains, blue light again had a strong inhibitory effect in comparison with white light. After-ripened Bd21-3 grains displayed almost full germination under all conditions, including under blue light (Fig. 7).

Red and far-red effects

In contrast to barley, red and far-red lights strongly influenced *Brachypodium* dormancy and germination. To explore the ability of red light to promote germination, dormant Bd18-1 grains (near 0% germination in light and darkness) were imbibed in the dark for 24 h, and then exposed for 1 h, 1, 5, or 10 d to red light. After these treatments, grains were returned to the dark, and germination recorded 10 d after imbibition (Fig. 8). While grains exposed to 10 d of continuous red light reached *c.* 60% germination, the 1-h or 1-d treatments had very little effect and only 3% germination was reached in both cases. Five days of continuous red light produced only *c.* 15% germination.

Red and far-red wavelengths are known to have a critical role in grain germination in many species via the action of members of the phytochrome (PHY) family of photoreceptors (reviewed in Franklin & Quail, 2010). Based on the observed effects of red and far-red light described above, the simplest explanation is that a light stable phytochrome, most likely PHYB (Franklin & Quail, 2010), can regulate germination in *Brachypodium*. To test

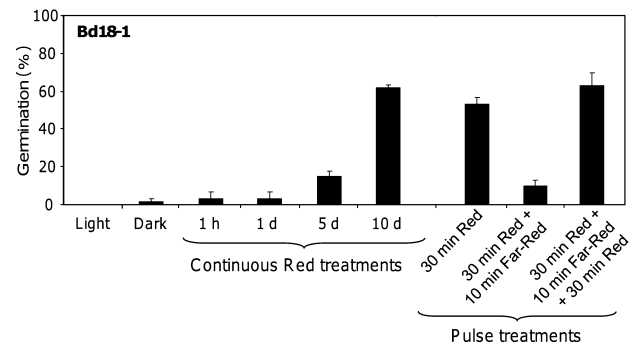

Fig. 8 Red and far-red effects on germination in *Brachypodium*. Dormant Bd18-1 grains were imbibed in water and then different red and far-red treatments were given. Germination was scored at day 10. Measurements are averages of three replicates with error bars representing SE of the mean.

this hypothesis, pulses of red and far-red light were used in various combinations, and germination again assessed after 10 d (Fig. 8). Daily red pulses of 30 min strongly promoted germination (to > 50%). However, if the red pulses were immediately followed by a 10-min pulse of far-red germination was strongly inhibited (< 10%). Finally, daily pulses given in the order red/far-red/red restored germination to the level observed after red pulses only.

Time-course of ABA during imbibition

To examine a possible correlation between ABA concentration and the degree of dormancy, the ABA content of Bd21-3 and Bd18-1 freshly-harvested (dormant) and after-ripened grains were quantified. Abscisic acid was extracted from dry grains and from grains imbibed for 1–4 d, under continuous white light and in darkness. For Bd21-3, the ABA content in dry dormant and after-ripened grains was the same (*c.* 450 ng g^{-1} FW). This initial ABA concentration decreased considerably over 1 d of imbibition in both dormant and after-ripened grains. However, the reduction of ABA was larger in after-ripened than in dormant grains, and by day 4 dormant grains contained *c.* 300 ng g^{-1} while after-ripened grains contained only *c.* 100 ng g^{-1} (Fig. 9a). The presence of light during imbibition had very little effect on grain ABA concentration (Fig. 9a), even though it clearly affected germination: the batch of Bd21-3 grains used in this experiment displayed 35% germination in the light and 66% in darkness when freshly-harvested, and 72% in light and 100% in darkness when after-ripened.

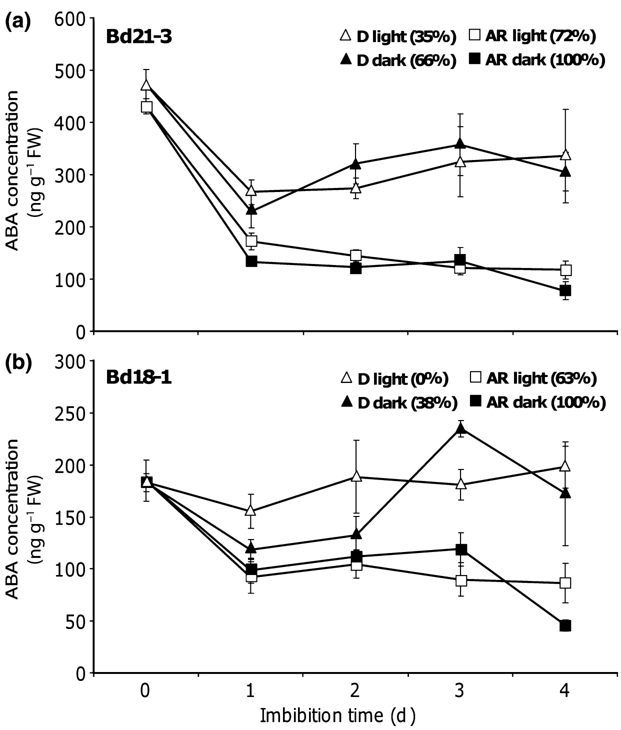

Fig. 9 Quantification of ABA during hydration in *Brachypodium*. The ABA content was quantified in embryos from dormant (D) and after-ripened (AR) Bd21-3 (a) and Bd18-1 (b) grains, imbibed in darkness or under continuous white light. Measurements are averages of three replicates with error bars representing SE of the mean. Germination rates of the different treatments are given in brackets.

The amount of ABA in Bd18-1 dormant dry grains was also the same as in after-ripened dry grains, *c.* 200 ng g^{-1} (half of the amount found in Bd21-3). After 1 d of imbibitions, the ABA content was reduced in after-ripened grains, and the concentrations remained very low until day 4, especially in the grains imbibed in darkness (Fig. 9b). However, in dormant Bd18-1 grains the ABA profiles after imbibition were very different. Dormant grains imbibed in light did not show a reduction in ABA concentrations which, instead, remained high throughout the experiment. Dormant grains imbibed in darkness displayed an initial reduction in ABA, but after day 2, ABA increased reaching *c.* 250 ng g^{-1} by day 3 (Fig. 9b). The Bd18-1 grains used in this experiment displayed 0% germination in the light and 38% in darkness when freshly-harvested, and 63% in light and 100% in darkness when after-ripened.

Expression of ABA metabolic genes

The dormant line Bd18-1 was selected for analysis of the expression of some key genes related to ABA metabolism. The *Brachypodium* genome was searched for sequences related to the *9-cis epoxycarotenoid dioxygenase* (*NCED*) and *CYP707A ABA 8′-hydroxylase* (*ABA8′OH*) gene families, as they are key enzymes in ABA biosynthesis and catabolism, respectively. Two *NCED* genes and two *ABA8′OH* genes were identified (see the Materials and Methods section), and named *BdNCED1*, *BdNCED2*, *BdABA8′OH-1* and *BdABA8′OH-2*. The expression of these genes was analysed in dormant and after-ripened dry grains and in grains imbibed in darkness or in white light for 1–4 d (Fig. 10).

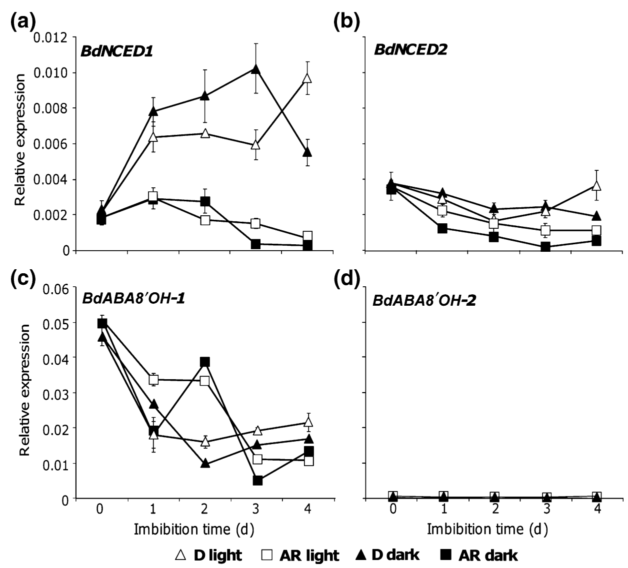

Fig. 10 Effect of white light and darkness on expression of ABA metabolic genes in embryos of dormant (D) and after-ripened (AR) *Brachypodium* Bd18-1 grains. Measurements are averages of three replicates with error bars representing SE of the mean. (a) *BdNCED1*, (b) *BdNCED2*, (c) *BdABA8′OH-1* and (d) *BdABA8′OH-2*.

BdNCED1 showed a dramatic change in expression between dormant and after-ripened grains (Fig. 10a). This gene was expressed at similar levels in dry dormant and after-ripened grain, but after imbibition its expression was strongly induced in dormant grains, but remained very low in after-ripened grains. During the first days of imbibition, dormant grains imbibed in darkness showed higher *BdNCED1* expression than dormant grains imbibed in light. However, after day 3 *BdNCED1* expression of the gene dropped significantly in grains imbibed in darkness but reached the maximum level in grains imbibed under continuous light. *BdNCED2* appeared to be less abundant than *BdNCED1* during imbibition, and was expressed at the same level in dormant and after-ripened dry grains (Fig. 10b). Although expression was not greatly induced after imbibition or dramatically affected by light, *BdNCED2* was expressed at slightly higher levels in dormant compared with after-ripened grains.

The expression of *BdABA8′OH-1* (Fig. 10c) was much higher than that of *BdABA8′OH-2* (Fig. 10d), suggesting that the ABA8′OH activity in embryos of imbibed grains is likely to be predominantly derived from *BdABA8′OH-1.* In dry dormant and after-ripened grains, *BdABA8′OH-1* expression was similar in all treatments (Fig. 10c). After imbibition, the expression of this gene was rapidly reduced in dormant grains and remained low for the rest of the time-course. In after-ripened grains, the expression of *BdABA8′OH-1* was also reduced after 1 d of imbibition, especially under dark conditions, but by day 2, expression was more than threefold higher in after-ripened than in dormant grains, in both darkness and continuous light. After day 2 the expression of *BdABA8′OH-1* in after-ripened grains decreased and differences from dormant grains were minimal.

Discussion

Coleorhiza and embryo anatomy and behaviour during germination

Brachypodium grains share many features with grains from other grasses (Brown & Morris, 1890; Merry, 1941). A detailed study of endosperm development in *Brachypodium* has recently been reported (Opanowicz *et al.*, 2011) revealing broad similarities with other cereals but also some interesting differences. In this paper we have focused on the anatomy of the embryo. Distinctive features of *Brachypodium* embryos include the bright yellow pigment in the coleorhiza and the prominent epiblasts, which are ontogenetically related to the coleorhiza (Brown, 1959; Foard & Haber, 1962). The dry embryo also displays regular folds, similar to dry embryos of wild oats (*Avena fatua*; Raju, 1985), and these are particularly prominent in the coleorhiza. As in barley, the seminal root breaks through the coleorhiza when coleorhiza growth ceases, and at the same time the coleorhiza epidermis produces epidermal hairs, seen also in barley and other species (Noda & Hayashi, 1960; Foard & Haber, 1962; Ozias-Akins & Vasil, 1983; Barrero *et al.*, 2009).

A further distinct feature of *Brachypodium* is the coleorhiza protrusion linking this tissue to the inner husk, which is superficially similar to structures protruding at the micropylar end of the germinating seed in pine (Tillman-Sutela & Kauppi, 2000). The coleorhiza tip protrusion and inner husk tissue also remain in contact in dormant barley (Barrero *et al.*, 2009), and may be a remnant of the nucellus at the micropylar end of the grain (Narayanaswami, 1956; Norstog, 1974; Suzuki *et al.*, 1991; reviewed in Sabelli & Larkins, 2009; cf. suspensor in pine embryos, Tillman-Sutela & Kauppi, 2000).

Cell separation in the coleoptile and coleorhiza is essential for germination and seedling emergence in grasses and related species (e.g. barley, Barrero *et al.*, 2009; rye, Hallam *et al.*, 1972; Sargent & Osborne, 1980). In *Brachypodium*, this process does not appear to commence during grain maturation as it does in barley (Barrero *et al.*, 2009), although the innermost coleorhiza cells next to the embryo root are the most elongated (Fig. 3c,d). Upon imbibition, cell elongation and separation commence throughout the coleorhiza and other tissues and, as seen in other cereals including barley (Sargent & Osborne, 1980), there is no cell division. This observation, together with the pronounced vacuolation of these cells, indicates that the coleorhiza is a terminally differentiated tissue with no potential for further meristematic activity and, although the epidermal cells are capable of producing hairs, it will undergo programmed cell death.

Natural variability for grain dormancy in *Brachypodium*

Seed dormancy is a very common and polymorphic trait in plants and even within the same species it can vary considerably between ecotypes, suggesting that seed dormancy is important for plant survival and adaptation. In grasses, grain dormancy has been reported in *c.* 50% of the genera that have been studied (Simpson, 1990). In *Brachypodium* species there are only a few reports on grain dormancy, all from an ecological perspective (Yan *et al.*, 2008). We have used six common *Brachypodium* inbred genotypes (Vogel *et al.*, 2006; Garvin *et al.*, 2008; Table 1) for a detailed description of the physiological and molecular characteristics of grain dormancy in this species. Our results show that there is great variability for grain dormancy between these genotypes. The genotypes used here are phylogenetically diverse (Vogel *et al.*, 2009) and apart from grain dormancy they also differ in other traits such as their vernalization requirements for flowering and grain size and shape (Fig. 1). We found stronger dormancy in Bd18-1 and Bd1-1, and weaker dormancy in Bd21-3 and Bd2-3. Under our conditions, white light was a dormancy-promoting factor in all *Brachypodium* genotypes examined, and grain dormancy was always weaker if grains were imbibed in darkness. In darkness, grain dormancy in all genotypes disappeared with a short after-ripening period (4 wk). By contrast, in light, grain dormancy lasted for a much longer time and in some genotypes more than a year of after-ripening was needed in order to break dormancy.

There is a vast literature on the effects of light on dormancy and germination in grasses, with a number of reports presenting conflicting results (reviewed in Simpson, 1990). Our observations of stronger dormancy expression under white light are in agreement with previous reports using other temperate grasses such as wild oat, barley and wheat (Cumming & Hay, 1958), thus indicating conservation of light regulation of dormancy in this group of grasses and *Brachypodium*.

Brachypodium also possesses both husk- and embryo-based dormancy, similar to temperate cereals such as barley, allowing both phenomena to be studied in this model system. Embryo-based dormancy is very strong in freshly-harvested grains, and removing the husk does little to promote germination. With after-ripening embryo-based dormancy disappears first and husk-based dormancy remains longer.

Light quality affects dormancy and germination in *Brachypodium*

The effects of light quality on grain dormancy and germination have been shown to be very important in grasses. Several studies have demonstrated a principal role for blue light acting as a germination repressor in barley (Chaussat & Zoppolo, 1983; Gubler *et al.*, 2008) and ryegrass (Goggin *et al.*, 2008), and other studies have shown a red and far-red effect in other species such wild oats (Hou & Simpson, 1992). After testing the effects of light of different spectral qualities we have found that *Brachypodium* was able to show both responses. Germination of *Brachypodium* grains was strongly inhibited by blue and far-red light, and was strongly promoted by red light. These effects were present in freshly-harvested grains of the dormant Bd18-1 genotype. In after-ripened Bd18-1 grains the inhibitory effect of blue light remained, but the red/far-red effects disappeared. Freshly-harvested grains from the low-dormancy genotype Bd21-3 behaved similarly to after-ripened Bd18-1 and blue light was able to inhibit germination, while red/far-red effects were undetectable. In after-ripened Bd21-3 grains the effect of blue light also disappeared and grains germinated strongly under all wavelengths.

The complexity of the light effects affecting the germination of *Brachypodium* grains suggests the involvement of at least two different families of photoreceptors, the red/far-red absorbing PHYs and the blue light absorbing cryptochromes (Banerjee & Batschauer, 2005). Wheat cryptochromes have been suggested to regulate the ABA signalling pathway, and have been shown to decrease germination when over-expressed in Arabidopsis (Xu *et al.*, 2009), consistent with a role in dormancy. In barley, blue light has been shown to directly induce the expression of the ABA biosynthetic gene *HvNCED1*, increasing the ABA content of imbibed grains (Gubler *et al.*, 2008). These results, together with the close phylogenetic relationship between *Brachypodium*, wheat and barley, suggest that the response of *Brachypodium* grains to blue light is also mediated by cryptochromes.

The observed red/far-red reversibility of grain germination in Bd18-1 suggests that the response to these wavelengths is controlled by the action of one or more light stable PHYs, consistent with previous suggestions for several grass species, including wild oats (Simpson, 1990). *Brachypodium* contains three PHY genes, named PHYA/B/C based on their similarity to PHY genes in other grasses (Higgins *et al.*, 2010). Based on detailed analysis in rice, and extensive work in model dicot systems (Takano *et al.*, 2009), PHYB is likely to be the sole PHY responsible for the observed effects on dormancy, although a role for PHYC cannot be ruled out. Our results demonstrate that relatively long periods of red light (up to 5 d) are considerably less effective at promoting germination than daily 30-min red pulses of the same intensity over 10 d, and similar observations have also been made for other grasses (Simpson, 1990). This cumulative red light response suggests that a threshold must be reached, over a number of days, before germination can occur, possibly ensuring that grains are in an optimal and stable environment.

Some early papers have discussed the ecological significance of light quality effects on dormancy and germination in some plant species. Hilton & Bitterli (1983) noted that the germination of freshly-shed *Avena fatua* grains from non-dormant ecotypes was inhibited by light; they did not germinate on the soil surface but could germinate if buried in the soil (in darkness). With after-ripening, the inhibitory effect of light disappeared and grains germinated equally well on the surface or incorporated into the soil. Freshly-shed *Avena* grains from dormant ecotypes did not germinate on the soil surface or when buried, and their germination was promoted by after-ripening. These observations have led to the hypothesis that the effect of the soil filtering the light spectrum changes dormancy. In general, long wavelengths such as far-red have a greater penetration in soil and short wavelengths such as blue are rapidly attenuated (Bliss & Smith, 1985). Depending on the soil composition, blue light would be totally attenuated in the first couple of millimetres, and the ratio of red/far-red light would decrease with depth (Bliss & Smith, 1985). This effect of soil filtering the light spectrum would provide, in theory, three different environments that could be very important for interpreting the light effects that we have seen in *Brachypodium* grains. On the soil surface a predominant blue light effect would inhibit germination. After the first few millimetres where blue light disappears, grains would find an appropriate germination environment as the ratio of red to far-red would be high. Finally, in a deeper environment, the ratio of red to far-red would be pushed towards far-red inhibiting germination again. As the after-ripening proceeds, red/far-red effects would disappear and the grains would germinate if they are buried, but on the surface germination would be delayed as the blue light effects last longer. Finally, with long after-ripening, the blue light effect would disappear and the grains would germinate on the surface, perhaps as a last chance for survival. This hypothesis may also be related to the domestication of cereals. Varieties that germinated better at variable depths would have been preferred to varieties that had more restricted germination characteristics.

It is interesting to note that in some domesticated cereals, including barley, red and far-red effects on dormancy and germination seem to be absent, and only blue light inhibition of germination has been reported (Gubler *et al.*, 2008). Given the close phylogenetic relatedness between barley and *Brachypodium*, it is possible that the domestication process has led to the loss of the PHY response in grains. This could have happened by the selection of genotypes with a similar behaviour to Bd21-3 and discarding those such as Bd18-1. If this hypothesis is correct, the reintroduction of PHY responses could provide important new opportunities for increasing grain dormancy in domesticated crops.

ABA content in dormant and after-ripened grains

The plant hormone ABA plays a critical role in dormancy in many species, and the amounts of ABA in imbibed dormant seeds are usually higher than the amounts found in imbibed after-ripened and non-dormant seeds (Millar *et al.*, 2006; Gubler *et al.*, 2008). The sensitivity to ABA is equally important for understanding the dormancy status of a grain, and dormant grains are usually more sensitive to ABA than after-ripened or nondormant grains (Barrero *et al.*, 2009). We have analysed the ABA content of dry and imbibed grains from the low-dormancy genotype Bd21-3 and from the high-dormancy genotype Bd18-1. In both genotypes the ABA content of after-ripened grains was less than that of dormant grains after 4 d of imbibition, consistent with an important role for ABA in regulating grain dormancy. However, Bd21-3 had a higher ABA content (two-fold difference) in dry grains than Bd18-1, indicating that ABA levels alone cannot be used to predict dormancy in different genotypes.

In barley the amounts of ABA during grain imbibition are also strongly correlated with the expression level of some key ABA metabolic genes (Millar *et al.*, 2006; Gubler *et al.*, 2008). The barley ABA biosynthetic gene *HvNCED1* has been shown to be induced by white and blue light and to a lesser extent repressed by after-ripening (Gubler *et al.*, 2008). By contrast, the ABA catabolic gene *HvABA8′OH-1* is induced by after-ripening, but is not affected by light (Gubler *et al.*, 2008). *Brachypodium* appears to differ from barley in that the expression of *BdNCED1* is strongly downregulated by after-ripening, and affected very little by white light. In the case of *BdABA8′OH-1*, expression was transiently induced in after-ripened grains and, like barley, light had little effect. These results imply that after-ripening of *Brachypodium* grains has a strong effect on the metabolism of ABA during early imbibition consistent with the reduced ABA levels found in after-ripened grains, and a role for ABA in regulating dormancy in this species. However, our results suggest that the effect of light on grain germination cannot be explained by changes in ABA metabolism or ABA levels. Other factors, such as the sensitivity to ABA or the role of other hormones such as gibberellins, may explain the observed effects of light on dormancy and germination in *Brachypodium*.

Brachypodium as a model for grain dormancy studies

The present study demonstrates the potential of *Brachypodium* as a model species for dissecting the mechanisms controlling grain dormancy in cereals. The *Brachypodium* grain is anatomically almost identical to the grains of barley and wheat. Different *Brachypodium* genotypes display great variability in grain dormancy, opening the possibility of constructing recombinant populations for mapping dormancy related genes. We have also analysed several factors with important effects on grain dormancy and germination, including the presence of the husk, the after-ripening process, light quality during imbibition, and ABA metabolism and levels. In general, these factors affect grain dormancy very similarly in *Brachypodium*, barley and wheat, making *Brachypodium* a good model for studying grain dormancy. Several differences between these grasses have been also identified, for example, the different effects of light quality on dormancy and ABA metabolism, potentially providing new approaches and tools for improving grain dormancy in cereals.

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References

Banerjee R, Batschauer A. 2005. Plant blue-light receptors. *Planta* **220**: 498–502.

Barrero JM, Jacobsen J, Gubler F. 2010. Seed dormancy: approaches for finding new genes in cereals. In: Pua EC, Davey MR, eds. *Plant developmental biology – biotechnological perspectives*. Berlin, Germany: Springer, 361–381.

Barrero JM, Talbot MJ, White RG, Jacobsen JV, Gubler F. 2009. Anatomical and transcriptomic studies of the coleorhiza reveal the importance of this tissue in regulating dormancy in barley. *Plant Physiology* **150**: 1006–1021.

Bliss D, Smith H. 1985. Penetration of light into soil and its role in the control of seed-germination. *Plant, Cell & Environment* **8**: 475–483.

Brown WV. 1959. The epiblast and coleoptile of the grass embryo. *Bulletin of the Torrey Botanical Club* **86**: 13–16.

Brown HT, Morris GH. 1890. Researches on the germination of some of the Gramineae. *Journal of the Chemical Society of London (Transactions)* **57**: 458–528.

Chang S, Puryear J, Cairney K. 1993. A simple and efficient method for isolating RNA from pine trees. *Plant Molecular Biology Reporter* **11**: 113–116.

Chaussat R, Zoppolo J. 1983. Lumière et germination de l'orge. *Bios* **14**: 30–32.

Cumming BG, Hay JR. 1958. Light and dormancy in Wild Oats (*Avena fatua* L). *Nature* **182**: 609–610.

Draper J, Mur LAJ, Jenkins G, Ghosh-Biswas GC, Bablak P, Hasterok R, Routledge APM. 2001. *Brachypodium distachyon*. A new model system for functional genomics in grasses. *Plant Physiology* **127**: 1539–1555.

Finch-Savage WE, Leubner-Metzger G. 2006. Seed dormancy and the control of germination. *New Phytologist* **171**: 501–523.

Foard DE, Haber AH. 1962. Use of growth characteristics in studies of morphologic relations. 1. Similarities between epiblast and coleorhiza. *American Journal of Botany* **49**: 520–523.

Franklin KA, Quail PH. 2010. Phytochrome functions in Arabidopsis development. *Journal of Experimental Botany* **61**: 11–24.

Garvin DF, Gu YQ, Hasterok R, Hazen SP, Jenkins G, Mockler TC, Mur LAJ, Vogel JP. 2008. Development of genetic and genomic research resources for *Brachypodium distachyon*, a new model system for grass crop research. *Crop Science* **48**: S69–S84.

Goggin DE, Steadman KJ, Powles SB. 2008. Green and blue light photoreceptors are involved in maintenance of dormancy in imbibed annual ryegrass (*Lolium rigidum*) seeds. *New Phytologist* **148**: 81–89.

Gu X-Y, Liu T, Feng J, Suttle JC, Gibbons J. 2010. The qSD12 undrlying gene promotes abscisic acid accumulation in early developing seeds to induce primary dormancy in rice. *Plant Molecular Biology* **73**: 97–104.

Gubler F, Hughes T, Waterhouse P, Jacobsen J. 2008. Regulation of dormancy in barley by blue light and after-ripening: effects on abscisic acid and gibberellin metabolism. *Plant Physiology* **147**: 886–896.

Gubler F, Millar AA, Jacobsen JV. 2005. Dormancy release, ABA and pre-harvest sprouting. *Current Opinion in Plant Biology* **8**: 183–187.

Hallam ND, Roberts BE, Osborne DJ. 1972. Embryogenesis and germination in rye (*Secale cereale* L.) 2. Biochemical and fine-structural changes during germination. *Planta* **105**: 293–309.

Harlan JR. 1975. *Crops and Man*. Madison, WI, USA: American Society of Agronomy.

Higgins JA, Bailey PC, Laurie DA. 2010. Comparative genomics of flowering time pathways using *Brachypodium distachyon* as a model for the temperate grasses. *PLoS ONE* **5**: e10065.

Hilton JR, Bitterli CJ. 1983. The influence of light on the germination of *Avena fatua* L (wild oat) seed and its ecological significance. *New Phytologist* **95**: 325–333.

Hong SY, Seo PJ, Yang MS, Xiang F, Park CM. 2008. Exploring valid reference genes for gene expression studies in *Brachypodium distachyon* by real-time PCR. *BMC Plant Biology* **8**: 112.

Hou JQ, Simpson GM. 1992. After-ripening and phytochrome action in seeds of dormant lines of wild oat (*Avena fatua*). *Physiologia Plantarum* **86**: 427–432.

Imtiaz M, Ogbonnaya FC, Oman J, van Ginkel M. 2008. Characterization of quantitative trait loci controlling genetic variation for preharvest sprouting in synthetic backcross-derived wheat lines. *Genetics* **178**: 1725–1736.

Merry J. 1941. Studies on the embryo of *Hordeum sativum* L. The development of the embryo. *Bulletin of the Torrey Botanical Club* **68**: 585–598.

Millar AA, Jacobsen JV, Ross JJ, Helliwell CA, Poole AT, Scofield G, Reid JB, Gubler F. 2006. Seed dormancy and ABA metabolism in Arabidopsis and barley: the role of ABA 8′-hydroxylase. *Plant Journal* **45**: 942–954.

Narayanaswami S. 1956. Structure and development of the caryopsis in some Indian millets. VI. *Setaria italica*. *Botanical Gazette* **118**: 112–122.

Noda A, Hayashi J. 1960. Studies on the coleorhiza of cereals. VII. On coleorhiza hairs in rice. *Nihon Sakumotsugaku Kai Kiji (Proceedings of the Crop Science Society of Japan)* **29**: 63–65.

Norstog K. 1974. Nucellus during early embryogeny in barley: fine structure. *Botanical Gazette* **135**: 97–103.

Opanowicz M, Hands P, Betts D, Parker ML, Toole GA, Mills ENC, Doonan JH, Drea S. 2011. Endosperm development in *Brachypodium distachyon*. *Journal of Experimental Botany* **62**: 735–748.

Ozias-Akins P, Vasil IK. 1983. Proliferation of and plant regeneration from the epiblast of *Triticum aestivum* (Wheat; Gramineae) embryos. *American Journal of Botany* **70**: 1092–1097.

Raju MVS. 1985. Water uptake and splitting of wild oat caryopsis. *Botanical Magazine Tokyo* **98**: 271–281.

Sabelli PA, Larkins BA. 2009. The development of endosperm in grasses. *Plant Physiology* **149**: 14–26.

Sargent JA, Osborne DJ. 1980. A comparative study of the fine structure of coleorhiza and root cells during the early hours of germination of rye embryos. *Protoplasma* **104**: 91–103.

Simpson GM. 1990. *Seed dormancy in grasses*. Cambridge, UK: Cambridge University Press.

Suzuki K, Taniguchi T, Maeda E. 1991. Scanning electron microscope observation of the morphological change in ventral organ of rice embryo during embryogenesis and germination. *Japanese Journal of Crop Science* **60**: 407–412.

Takano M, Inagaki N, Xie X, Kiyota S, Baba-Kasai A, Tanabata T, Shinomura T. 2009. Phytochromes are the sole photoperceptors for perceiving red/far-red light in rice. *Proceedings of the National Academy of Sciences, USA* **106**: 14705–14710.

The International Brachypodium Initiative. 2010. Genome sequencing and analysis of the model grass *Brachypodium distachyon*. *Nature* **463**: 763–768.

Tillman-Sutela E, Kauppi A. 2000. Structures contributing to the completion of conifer seed germination. *Trees* **14**: 191–197.

Vogel JP, Garvin DF, Leong OM, Hayden DM. 2006. *Agrobacterium*-mediated transformation and inbred line development in the model grass *Brachypodium distachyon*. *Plant Cell Tissue and Organ Culture* **84**: 199–211.

Vogel JP, Hill T. 2008. High-efficiency *Agrobacterium*-mediated transformation of *Brachypodium distachyon* inbred line Bd21-3. *Plant Cell Reports* **27**: 471–478.

Vogel JP, Tuna M, Budak H, Huo NX, Gu YQ, Steinwand MA. 2009. Development of SSR markers and analysis of diversity in Turkish populations of *Brachypodium distachyon*. *BMC Plant Biology* **9**: 88.

Xu P, Xiang Y, Zhu HL, Xu HB, Zhang ZZ, Zhang CQ, Zhang LX, Ma ZQ. 2009. Wheat cryptochromes: subcellular localization and involvement in photomorphogenesis and osmotic stress responses. *Plant Physiology* **149**: 760–774.

Yan J, Cheng J, Wang T, Gutterman Y, Nevo E. 2008. Dormancy and germination of *Brachypodium distachyon* caryopses derived from Israel. *Journal of Tropical and Subtropical Botany* **16**: 104–108.

Ye H, Foley ME, Gu X-Y. 2010. New seed dormancy loci detected from weedy rice-derived advanced populations with major QTLs alleles removed from the background. *Plant Science* **179**: 612–619.